Form PTO-1390 (REV 10-94) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
480821.90043	
U.S APPLICATION NO (If known, see 37 C F R 1 5)	
09/720411	

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/02005	25 June 1999	26 June 1998

TITLE OF INVENTION: CALCIUM PHOSPHATE COATED VESICLES

APPLICANT(S) FOR DO/EO/US: CZERNUSZKA, Jan Tadeusz; HADDOW, David Bryan

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371
3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1)
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b [X] has been transmitted by the International Bureau.
   c [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [] A translation of the International Application into English (35 U.S.C 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c [X] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3 31 in included
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information: Copy of Form PCT/IB/308, Notice Informing Applicant of Communication of International Application to Designated Offices; Postcard Receipt

EXPRESS MAIL NO. EK950628036US

QB

526 Rec'd PCT/PTO 21 DEC 2000

U.S. APPLICATION NO (if known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/720411	PCT/GB99/02005	480821.90043

17. [X] The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....$1000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... 860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... 710.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of pCT Article 33(1)-(4)... .... 690.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)... ..... 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	33 -20 =	13	X $18.00	$104.00	
Independent claims	2 -3 =	0	X $78.00	$0.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)	6		X $260.00	$0.00	

TOTAL OF ABOVE CALCULATIONS =	$964.00	
[] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.	$	
SUBTOTAL =	$964.00	
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.429(f)). +	$	
TOTAL NATIONAL FEE =	$964.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +	$	
TOTAL FEES ENCLOSED =	$964.00	
	Amount to be: refunded	$
	charged	$

a. [ ] A check in the amount of $_____ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No 17-0055 in the amount of $964.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 17-0055. A duplicate copy of this sheet is enclosed

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

SIGNATURE Jean C. Baker

NAME Jean C. Baker

REGISTRATION NUMBER Reg. No. 35,433

QB

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	**CZERNUSZKA**	Docket No.:	480821.90043
Serial No.:	**Unassigned**	Filed:	**Concurrently herewith**
Int'l appln No.:	**PCT/GB99/02005**	Int'l filing date:	**25 June 1999**
Title:	**CALCIUM PHOSPHATE COATED VESICLES**		

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PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In connection with the above-identified application filed herewith, please enter the following preliminary amendment:

IN THE CLAIMS:

Please amend claims 5, 6, 7, 10, 13, 16, 17, 18, 20, 21, 24, 25, 26, 30, 32 and 33 as follows:

CLAIMS

5. A droplet according to claim 3 [or claim 4] wherein the surfactant is an anionic surfactant.

6. A vesicle or droplet according to [any one of the preceding claims] claim 1 wherein the outer layer further comprises ions selected from carbonate, hydrogen phosphate, chloride, fluoride or magnesium.

7. A vesicle or droplet according to [any one of the preceding claims] claim 1 wherein the thickness of the outer layer is from 5 to 50 nm.

10. A vesicle or droplet according to [any one of the preceding claims] claim 1 wherein the size of the vesicle or droplet is from 100 nm to 10 μm.

13. A vesicle or droplet according to [any one of the preceding claims] claim 1 which further comprises a pharmaceutically active compound.

16. A process for preparing a vesicle as claimed in [any one of claims 1, 2 or 6 to 15] claim 1, which process comprises

a) forming a vesicle in an aqueous mixture comprising a phospholipid, and

b) calcifying the vesicle by contacting said vesicle with an aqueous solution comprising calcium and phosphate ions.

17. A process for preparing a hydrophobic droplet as defined in [any one of claims 3 to 15] claim 1 which process comprises

a) forming a hydrophobic droplet in an aqueous mixture comprising a hydrophobic liquid or solid and a surfactant, and

b) calcifying the droplet by contacting said droplet with an aqueous solution comprising calcium and phosphate ions.

18. A process according to claim [16 or claim] 17 wherein the aqueous mixture further comprises an alcohol.

20. A process according to claim 18 [or claim 19] wherein the concentration of alcohol is no more than 10% by volume of the aqueous mixture.

21. A process according to [any one of claims 16 to 20] claim 16 wherein the ratio of calcium to phosphate ions in the aqueous solution is from 1:1 to 2:1.

24. A vesicle according to claim 1 prepared by the process according to [any one of claims 16 or 18 to 23] claim 16.

25. A droplet according to claim 3 prepared by the process according to [any one of claims 17 to 23] claim 17.

26. A solid substrate wherein regions of said substrate have attached thereto a layer comprising vesicles or droplets as claimed in [any one of claims 1 to 15, 24 or 25] claim 1 with other region or regions having no vesicles or droplets attached thereto.

30. A process for preparing a substrate according to [any one of claims 26 to 29] claim 26 which process comprises electrolytically depositing the coating comprising vesicles or droplets onto the conducting regions of the substrate.

32. A substrate according to [any one of claims 26 to 29] claim 26 for use in the treatment of the human or animal body.

33. Use of a substrate according to [any one of claims 26 to 29] claim 26 in the manufacture of a medically acceptable implant the treatment of bone disorders or in the delivery of pharmaceutically active compounds.

Remarks

The above amendments are being made to eliminate multiple dependencies in the claims of this application.

No fee is believed necessary to enter this amendment. However if a fee is necessary, please charge Deposit Account 17-0055.

Applicant respectfully requests that the preliminary amendment described herein be entered into the record prior to examination and consideration of the above-identified application.

QUARLES & BRADY LLP

By: Jean C. Baker

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Date: December 21, 2000

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CALCIUM PHOSPHATE COATED VESICLES

The present invention relates to coating materials comprising calcium phosphate, processes for their preparation and their use in coatings. The invention in particular relates to vesicles or hydrophobic droplets comprising an outer layer which comprises calcium phosphate, their preparation and their use in, for example, the coating of implants and drug delivery.

Porous monolithic ceramics based on hydroxyapatite (HA) have been shown to aid in osteoconduction of bone when implanted into a bony defect. However, they are too brittle to use in structural applications.

One way to overcome this problem is to coat a metallic implant with HA. The HA provides the bone bonding capacity while the metal provides the structural support. Unfortunately, many common methods for producing coatings require elevated temperatures which result in undesired effects such as degradation of HA to various calcium phosphate phases depending on the stoichiometry of the starting powder and the cooling rate on coating formation [R. LeGEROS, *Clinical Materials.* **14** (1993) 65].

Implant materials may be plasma sprayed with hydroxyapatite prior to being exposed to the biological environment. However, use of plasma-sprayed coatings [K. de GROOT, *J. Biomed. Mat. Res.* **21** (1987) 1375] has shown that high processing temperatures are responsible for the drawbacks experienced with such coatings. The drawbacks include, for example, variable compositions, lack of control over the microstructure, and cracking and delamination of the coating from the substrate. The last two effects also arise due to the relative thickness of the coating resulting in poor mechanical properties across the coating thickness. In addition, plasma-sprayed coatings offer none of the porosity required to encourage bone ingrowth. They also require expensive processing equipment.

In order to utilise a wider range of substrates than the metals traditionally used, it is desirable to be able to use a coating method that does not require elevated processing temperatures. This would allow the use of materials that are traditionally considered bioinert, but which may have the necessary strength and toughness to act as implant materials. Several such methods exist, and include a biomimetic process

[T. KOKUBO, in "Bone-Bonding Biomaterials" (Reed Healthcare Communications, Netherlands, 1992) 102] and electrophoretic deposition [M. SHIRKANZADEH, M. AZADEGAN, V. STACK and S. SCHREYER, *Materials Letters.* **18** (1994) 211]. However, these methods are substrate specific, and in the case of the biomimetic process the time period required for apatite growth is significant.

It is also advantageous to incorporate a degree of porosity into the coatings to encourage bone ingrowth in an implant situation. To achieve such ingrowth the porosity must be on a scale compatible with bone regeneration.

The present invention provides coating materials comprising calcium phosphate which overcome the problems discussed above. They may be used to coat substrates at low temperatures while offering a high degree of control over both the coating thickness and the degree of porosity. Such a low temperature method also allows the incorporation into the coating of other compounds which would undergo degradation at high temperatures such as, for example, heat sensitive pharmaceutically active compounds.

In a first aspect, the present invention provides a vesicle comprising

a) an inner layer which comprises a phospholipid, and

b) an outer layer which comprises calcium phosphate.

The phospholipid comprising the inner layer is any phospholipid capable of forming vesicles in an aqueous mixture, or a mixture of such phospholipids. Preferably the phospholipid is L-α-phosphatidylserine. More preferably the phospholipid is L-α-phosphatidylcholine.

The outer layer may further comprise other ions which can be incorporated to modify the properties of the calcium phosphate including anions such as carbonate, hydrogen carbonate, hydrogen phosphate, chloride and fluoride and cations such as magnesium.

The vesicles of the present invention may contain pharmaceutically active compounds including compounds which assist the binding of the coating to existing bone (bone growth factors), treat a specific bone disease or any diseased region adjacent to bone, or relieve pain. In particular, the vesicles of the present invention may contain compounds for the treatment of tumours such as ^{32}P or ^{89}Sr containing

compounds, compounds for the reduction of pain arising from tumours such as narcotic analgesics (which may be administered in lower doses according to the invention as they may be administered at the site of the tumour), compounds for the reduction of osteoclast activity caused by tumour cells such as indomethacin, prostoglandins and interleuken 6 inhibitors as well as those compounds which treat specific bone diseases such as osteoporosis, for example, parathyroid hormone, vitamin D derivatives, bisphosphanates, bone morphogenetic proteins and antibiotics, or mixtures thereof.

It has been found that the vesicles of the present invention may be readily obtained by forming them in an aqueous mixture comprising a phospholipid and then calcifying said vesicles by contacting them with an aqueous solution comprising calcium and phosphate ions.

The phospholipid concentration in the aqueous mixture should be below the concentration at which agglomeration of the phospholipid may occur. Preferably the phospholipid concentration is from 5×10^{-5} to 1×10^{-3}g per cm^3, more preferably from 5×10^{-5} to 7.5×10^{-4}g per cm^3 and most preferably from 2.5×10^{-4} to 5×10^{-5}g per cm^3.

The vesicles can be formed by agitating the aqueous mixture comprising a phospholipid as described above. This can be achieved by stirring but preferably the mixture is agitated by high-frequency sound waves having a frequency and power sufficient to form an emulsion. Preferably the frequency of sonication is from 20 to 30 kHz.

The temperature of agitation should be below the boiling point of the aqueous mixture. It should also be below the temperature at which degradation of the phospholipid may occur. Preferably the temperature of agitation is below 70°C, more preferably it is below 50°C and most preferably it is about room temperature.

The agitation time is dependent upon the method of agitation and the concentration of the aqueous mixture. Preferably the mixture is agitated for a time sufficient to form an emulsion or until no further vesicles are formed i.e. a steady state is reached. If the mixture is sonicated the agitation time for a mixture with a concentration of 5×10^{-5}g of phospholipid per cm^3 of aqueous mixture is generally

from 15 minutes to 2 hours, especially about 1 hour. If a mixture of the same concentration is stirred to form the vesicles then 30 minutes to 4 hours, more preferably 1 hour to 3 hours and most preferably about 2 hours.

One or more alcohols may be incorporated into the aqueous mixture to increase vesicle size in accordance with known methods. The alcohol is typically methanol, ethanol, propanol or butanol. Preferably the alcohol is ethanol. The concentration of the alcohol is generally below the concentration at which the phospholipid begins to dissolve. Preferably the alcohol concentration is no more than about 10% by volume of the aqueous mixture.

Other components which may be added to the aqueous mixture include, for example, surfactants and pharmaceutically active compounds. Preferred surfactants include anionic surfactants, for example esters of carboxyls, sulphates and phosphates. Use of surfactants can reduce the agitation time required to produce the vesicles.

Settling of the vesicles after agitation may be prevented by, for example, magnetically stirring using a low shearing rate.

Calcification of the vesicles can be carried out by contacting the phospholipid vesicles with an aqueous solution comprising calcium and phosphate (PO_4^{3-}) ions. Typically the ratio of calcium to phosphate ions is from 1:1 to 2:1, preferably from 1.4:1 to 2:1 and more preferably about 1.5:1.

The source of calcium ions in the solution is any water soluble organic or inorganic calcium compound, preferably calcium chloride or calcium nitrite and more preferably calcium nitrate.

The source of phosphate ions in the solution is any water soluble phosphate compound, preferably an orthophosphate, for example, a potassium orthophosphate, especially di-potassium hydrogen orthophosphate trihydrate.

As indicated above, other ions may be incorporated into the layer comprising calcium phosphate. For example, carbonate and hydrogen phosphate ions may be added to increase the resorption rate in the body whereas chloride, fluoride and magnesium ions may be added to decrease the resorption rate.

In particular, carbonate ions may be added to the aqueous solution of calcium

and phosphate ions to vary the crystallinity and stoichiometry of the calcified layer. The maximum concentration of carbonate ions will depend on pH, temperature and the presence of other ions. It will be appreciated, though, that the calcified layer is preferably a calcium phosphate layer or a substituted calcium phosphate layer. The source of carbonate ions is any soluble carbonate or hydrogen carbonate compound and is preferably potassium hydrogen carbonate or sodium hydrogen carbonate.

Calcification may be performed by simultaneously contacting the vesicles with calcium and phosphate ions or by introducing the vesicles into a calcium solution, for example, for about two hours, prior to the addition of phosphate ions.

The time for which the vesicles are contacted with the calcifying solution affects the thickness of the outer layer formed on the vesicles. Typically after about 1 hour the thickness of the layer is about 10 nm. The thickness of the layer (coupled with its porosity) may affect the rate at which the vesicles are broken down in the body and the rate of release of any pharmaceutically active compounds from within the vesicles. Preferably the coat thickness is from 5 to 50 nm, more preferably about 5 to 20 nm and most preferably about 10 nm.

In another aspect, the present invention provides a hydrophobic droplet comprising

a) a hydrophobic core,

b) an inner layer which comprises a surfactant, and

c) an outer layer which comprises calcium phosphate.

The hydrophobic core preferably comprises a solid or liquid hydrocarbon or lipid. It may further comprise water insoluble pharmaceutically active compounds.

The surfactant may be any surfactant which can reduce the surface energy of the non-aqueous droplet. It may also provide an active site for calcium phosphate deposition. Preferably the surfactant is an anionic surfactant, for example an ester of a carboxyl, sulphate or phosphate.

It has been found that the hydrophobic droplets of the present invention may be readily obtained by forming hydrophobic droplets in an aqueous mixture comprising a hydrophobic liquid or solid and a surfactant. The droplets are then calcified by contacting them with an aqueous solution comprising calcium and

phosphate ions and, optionally, other ions, as discussed above.

The hydrophobic droplets of the present invention may be formed by agitation and subsequent calcification as described above.

The size of the vesicles and hydrophobic droplets of the present invention is generally from 100 nm to 10 μm, preferably at least 300 nm and more preferably at least 1 μm. Size may be increased to over 1 μm by addition of an alcohol, for example, ethanol, to the aqueous mixture prior to agitation. The size of the vesicles or droplets may also be controlled by extrusion processes using, for example, hypodermic syringes or porous membranes.

In a further, aspect the present invention provides a solid substrate wherein regions of said substrate have attached thereto a layer comprising the vesicles or droplets described above with other region or regions having no vesicles or droplets attached thereto. Such substrates may find application in the treatment of bone disorders and/or the *in vitro* delivery of pharmaceutically active compounds.

The substrates which can be coated may be electrically conductive over all or part of their surface. They may be, for example, metals such as gold, plastics or ceramics coated with metal over all or part of their surface, metals partially coated with plastic, or semi-conductors. Preferably the substrates have non-conducting regions on their surfaces of from 10 μm to 2 mm in diameter and more preferably the regions are about 150 μm in diameter.

The substrates may be coated using an electrolytic deposition process or by applying vesicles or droplets of the present invention in the form of a powder. Preferably the substrates are electrolytically coated.

The electrolytic deposition process may be carried out in an aqueous solution at a pH of from 5 to 11, preferably 6 to 8, more preferably about 7.4. The form of the calcium phosphate deposited may vary with pH. For example, at high pH hydroxyapatite may be deposited whereas at low pH brushite may be deposited.

The temperature of deposition is generally below 100°C; preferably below 70°C and more preferably about 50°C.

A salt such as, for example, potassium chloride may be added to the solution to maintain supersaturation by keeping a high background ionic strength and act as

an electrolyte. Alternatively, calcium and phosphate can be added during the precipitation process to maintain supersaturation.

The coating thickness does, of course, increase with deposition time. For example, after a deposition time of 1 hour the coating thickness is about 2 μm. Multiple depositions may be performed or deposition time prolonged to access thicker coatings, for example coatings of about 20 μm.

Coatings may be formed from mixtures of the vesicles and/or droplets of the present invention. Thus different regions on the surface of the substrates may be coated with different types of vesicles or droplets. For example, a non-conducting pattern may be applied to the substrate prior to the first deposition. After the first deposition using one or more types of vesicle and/or droplet of the present invention the non-conducting pattern may be removed and a second deposition performed using different vesicles and/or droplets according to the invention. Alternatively, for example, a metal substrate is coated. It may then be subjected to a partial etching or lithographic process and a second deposition performed in a different solution of vesicles and/or droplets. Use of a variety of vesicles and/or droplets may allow the release of pharmaceutically active compounds in the coating to be controlled. For example, compounds incorporated into vesicles or droplets with a thin coating will be released more rapidly than compounds incorporated into vesicles or droplets with a thick coating.

The present invention is further illustrated, merely by way of example, with reference to the figures in which:

Figure 1 shows a schematic of the experimental apparatus used for electrolytic deposition of the vesicles and droplets of the present invention onto the surface of substrate plates.

Figure 2 shows an X-ray diffraction trace of calcified vesicles formed in a calcification solution comprising carbonate ions at atmospheric concentration. The trace shows peaks diagnostic of HA.

Figure 3 shows a schematic illustration of the structural hierarchy and graded porosity in the coatings of the present invention. It will be appreciated that the first order porosity concerns the spacing between the deposits, the second order porosity

concerns the porosity of the vesicles before calcification and the third order porosity concerns the porosity of the calcium phosphate layer. As discussed above all of these three can be varied by appropriate manipulation of the process parameters.

The Examples which follow further illustrate the present invention with reference to the figures.

Examples

Coatings were examined using a Jeol 840 FEG SEM using an accelerating voltage of 2 kV. Phase determination was carried out on a Phillips PW 1710 X-ray diffractometer. Fourier Transform Infra-Red spectroscopy was performed on an ATI Mattson Genesis Series Spectrometer in the wavenumber range 4000 to 400 cm^{-1}.

Vesicle Formation

5 or 10 mg or L-α-Phosphatidycholine or L-α-Phosphatidylserine (both from Sigma, U.K.) were sonicated (i.e., agitated by high-frequency sound waves) using a Kerry Ultrasonics Ltd ultrasonic stirrer in 20 ml of distilled water for up to one hour at room temperature to form spherical vesicles. After sonication settling of the vesicles was prevented by magnetic stirring using a low shearing rate. These phospholipids are well known to show a high affinity for calcium binding.

Calcification of Vesicles

The sonicated solution was added to a calcium phosphate (CaP) working solution. This working solution was made up as follows: solutions of 1.75 mM [Ca] were made up of Ca/P ratio=5/3 using analytical grade calcium nitrate tetrahydrate, $Ca(NO_3)_2.4H_2O$ and di-potassium hydrogen orthophosphate trihydrate, $K_2HPO_4.3H_2O$ (both from Aldrich, U.K.) in distilled water. Carbonate ions were incorporated using potassium hydrogen carbonate, $KHCO_3$ (from Adrich, U.K.). Carbonate ions were added to correspond to the following concentrations: (i)

atmospheric CO_2 (1.445×10^{-7} M), and (ii) physiological [CO_3^{2-}] (1.6×10^{-6} M).

Deposition of Vesicles

A platinum anode and a 304 stainless steel cathode were placed in the supersaturated solutions prepared as described above. The electrodes were connected to a Phillips PM2831 programmable power supply capable of both constant current (CC) and constant voltage (CV) characteristics. Calcium ion concentration, pH and temperature were monitored using an ION85 meter (Radiometer Ltd. Copenhagen), from which the supersaturation was measured. All experiments were performed at 50°C, starting pH 7.4, working solution 1.75 mM [Ca^{2+}], 0.1M KCl, with a working electrode spacing of 2.25 cm and 2.2V applied potential. As well as the lipid/water solutions, additional solutions were prepared incorporating an alcohol (ethanol) into the lipid solution to be sonicated. Calcification and deposition of the vesicles was performed in the same manner as previously described. At the deposition temperature of 50°C, the alcohol slowly evaporates, resulting in an increased vesicle size. Because phospholipids are highly soluble in alcohols, the percentage volume of alcohol in the sonicated solution was limited to 10% (2 ml). The experimental set-up is detailed in Figure 1.

There was no change in the solution as the temperature was raised from the vesicle preparation temperature (ambient room temperature) up to the deposition temperature of 50°C. Because the vesicles were formed before introduction into a calcium containing media, all calcification occurred on the outer surface of the vesicles. This was confirmed by SEM examination of the coatings.

XRD and FT-IR of coatings prepared with a carbonate concentration equal to atmospheric equilibrium indicated that the deposits comprised near stoichiometric hydroxyapatite, with very low levels of carbonate. These did not show up in the XRD traces (Figure 2) but peaks attributable to CO_3^{2-} were seen in the FT-IR spectra. At higher carbonate concentrations the XRD traces were similar, but the carbonate bands in the FT-IR spectra were more intense.

SEM investigations of coatings prepared using 5 mg phospholipid indicated

that surface coverage was typically less than 50%. Coatings prepared from 10 mg lipid solutions exhibited greater coverage such that the substrate was not visible under SEM. Phosphatidylcholine coatings were chosen for examination under the Jeol FEG-SEM because a comparison of five of these coatings with those prepared using phosphatidylserine showed that % cover was greater with the phosphatidylcholine.

SEM of phosphatidylcholine (10 mg) coatings showed that the vesicle size ranged from 300 nm for aqueous solutions, up to 1 μm for solutions incorporating alcohol. It is thus possible to vary the vesicle size by choice of solutions properties.

The calcified wall thickness was determined to be about 10 nm, for a vesicle suspension exposed to calcium ions for a period of one hour. The calcified wall thickness increases with exposure time to calcium ions.

Coating thickness (i.e. after impingement of spheres onto the substrate) increases with deposition time. By varying the deposition time, coatings of the required thickness may be obtained.

CLAIMS

1. A vesicle comprising

a) an inner layer which comprises a phospholipid, and

b) an outer layer which comprises calcium phosphate.

2. A vesicle according to claim 1 wherein the phospholipid is selected from L-α-phosphatidylcholine and L-α-phosphatidylserine.

3. A hydrophobic droplet comprising

a) a hydrophobic core,

b) an inner layer which comprises a surfactant, and

c) an outer layer which comprises calcium phosphate.

4. A droplet according to claim 3 wherein the hydrophobic core comprises a solid or liquid hydrocarbon or lipid.

5. A droplet according to claim 3 or claim 4 wherein the surfactant is an anionic surfactant.

6. A vesicle or droplet according to any one of the preceding claims wherein the outer layer further comprises ions selected from carbonate, hydrogen phosphate, chloride, fluoride or magnesium.

7. A vesicle or droplet according to any one of the preceding claims wherein the thickness of the outer layer is from 5 to 50 nm.

8. A vesicle or droplet according to claim 7 wherein the thickness of the outer layer is from 5 to 20 nm.

9. A vesicle or droplet according to claim 8 wherein the thickness of the outer layer is about 10 nm.

10. A vesicle or droplet according to any one of the preceding claims wherein the size of the vesicle or droplet is from 100 nm to 10 μm.

11. A vesicle or droplet according to claim 10 wherein the size of the vesicle or droplet is at least 300 nm.

12. A vesicle or droplet according to claim 11 wherein the size of the vesicle or droplet is at least 1 μm.

13. A vesicle or droplet according to any one of the preceding claims

which further comprises a pharmaceutically active compound.

14. A vesicle or droplet according to claim 13 wherein the pharmaceutically active compound assists the binding of a coating comprising the vesicle or droplets to bone, treats a specific bone disease or any diseased region adjacent to bone, or relieves pain.

15. A vesicle or droplet according to claim 14 wherein the pharmaceutically active compound is selected from parathyroid hormone, vitamin D derivatives, bisphosphanates, bone morphogenetic proteins, analgesics, ^{32}P or ^{89}Sr containing compounds, indomethacin, prostoglandins, interleuken 6 inhibitors and antibiotics.

16. A process for preparing a vesicle as claimed in any one of claims 1, 2 or 6 to 15, which process comprises

a) forming a vesicle in an aqueous mixture comprising a phospholipid, and

b) calcifying the vesicle by contacting said vesicle with an aqueous solution comprising calcium and phosphate ions.

17. A process for preparing a hydrophobic droplet as defined in any one of claims 3 to 15 which process comprises

a) forming a hydrophobic droplet in an aqueous mixture comprising a hydrophobic liquid or solid and a surfactant, and

b) calcifying the droplet by contacting said droplet with an aqueous solution comprising calcium and phosphate ions.

18. A process according to claim 16 or claim 17 wherein the aqueous mixture further comprises an alcohol.

19. A process according to claim 18 wherein the alcohol is selected from methanol, ethanol, propanol and butanol.

20. A process according to claim 18 or claim 19 wherein the concentration of alcohol is no more than 10% by volume of the aqueous mixture.

21. A process according to any one of claims 16 to 20 wherein the ratio of calcium to phosphate ions in the aqueous solution is from 1:1 to 2:1.

22. A process according to claim 21 wherein the ratio of calcium to

phosphate ions is from 1.4:1 to 2:1.

23. A process according to claim 22 wherein the ratio of calcium to phosphate ions is about 1.5:1.

24. A vesicle according to claim 1 prepared by the process according to any one of claims 16 or 18 to 23.

25. A droplet according to claim 3 prepared by the process according to any one of claims 17 to 23.

26. A solid substrate wherein regions of said substrate have attached thereto a layer comprising vesicles or droplets as claimed in any one of claims 1 to 15, 24 or 25 with other region or regions having no vesicles or droplets attached thereto.

27. A substrate according to claim 26 which comprises

a) electrically conducting and non-conducting regions on its surface, and

b) a layer comprising vesicles or droplets on the conducting regions.

28. A substrate according to claim 27 wherein the non-conducting regions are from 10 μm to 2 mm in size.

29. A substrate according to claim 28 wherein the non-conducting regions are about 150 μm in size.

30. A process for preparing a substrate according to any one of claims 26 to 29 which process comprises electrolytically depositing the coating comprising vesicles or droplets onto the conducting regions of the substrate.

31. A substrate according to claim 26 prepared by the process according to ~~claim 30~~.

32. A substrate according to any one of claims 26 to 29 for use in the treatment of the human or animal body.

33. Use of a substrate according to any one of claims 26 to 29 in the manufacture of a medically acceptable implant the treatment of bone disorders or in the delivery of pharmaceutically active compounds.

Fig.1.

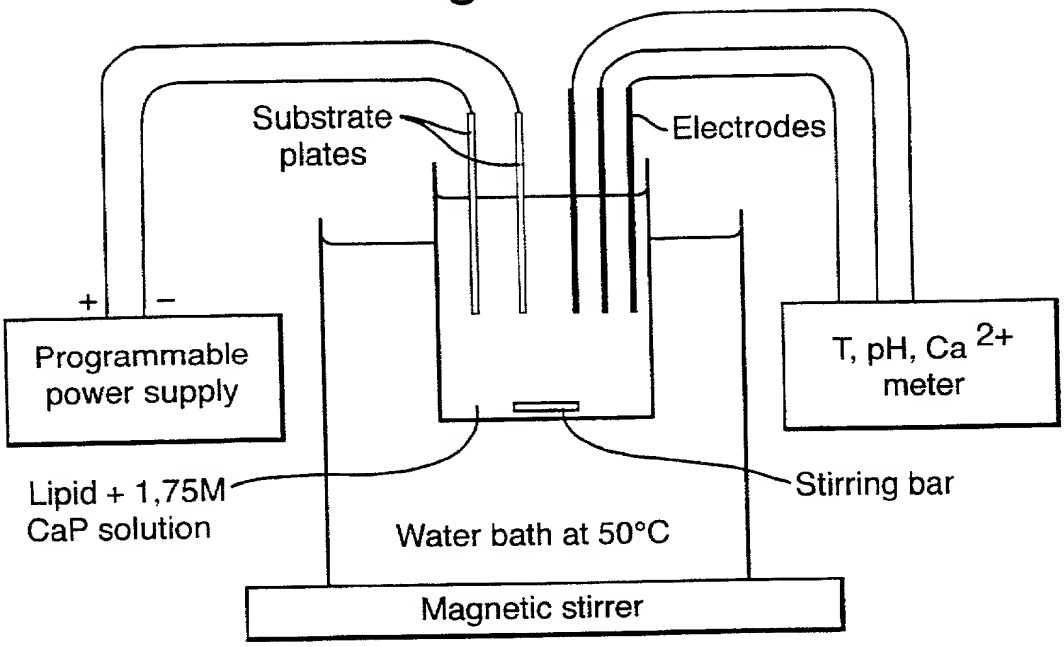
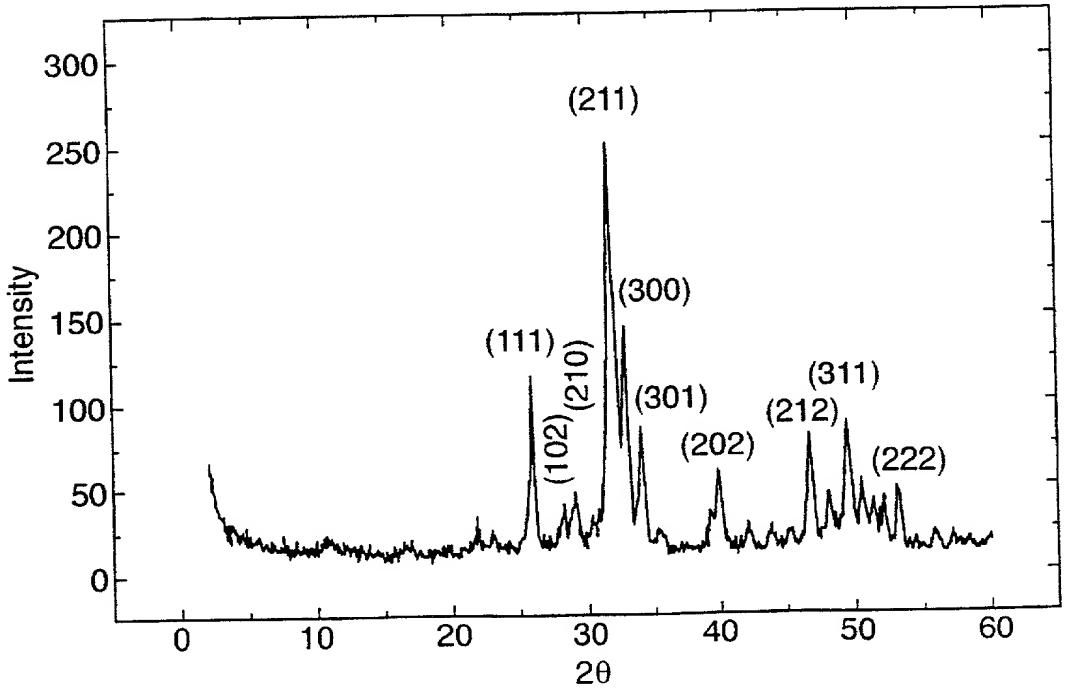

Substrate plates
Electrodes
+
−
Programmable power supply
T, pH, Ca 2+ meter
Lipid + 1,75M CaP solution
Stirring bar
Water bath at 50°C
Magnetic stirrer

Fig.2.


Intensity
2θ
(211)
(300)
(111)
(210)
(102)
(301)
(202)
(311)
(212)
(222)

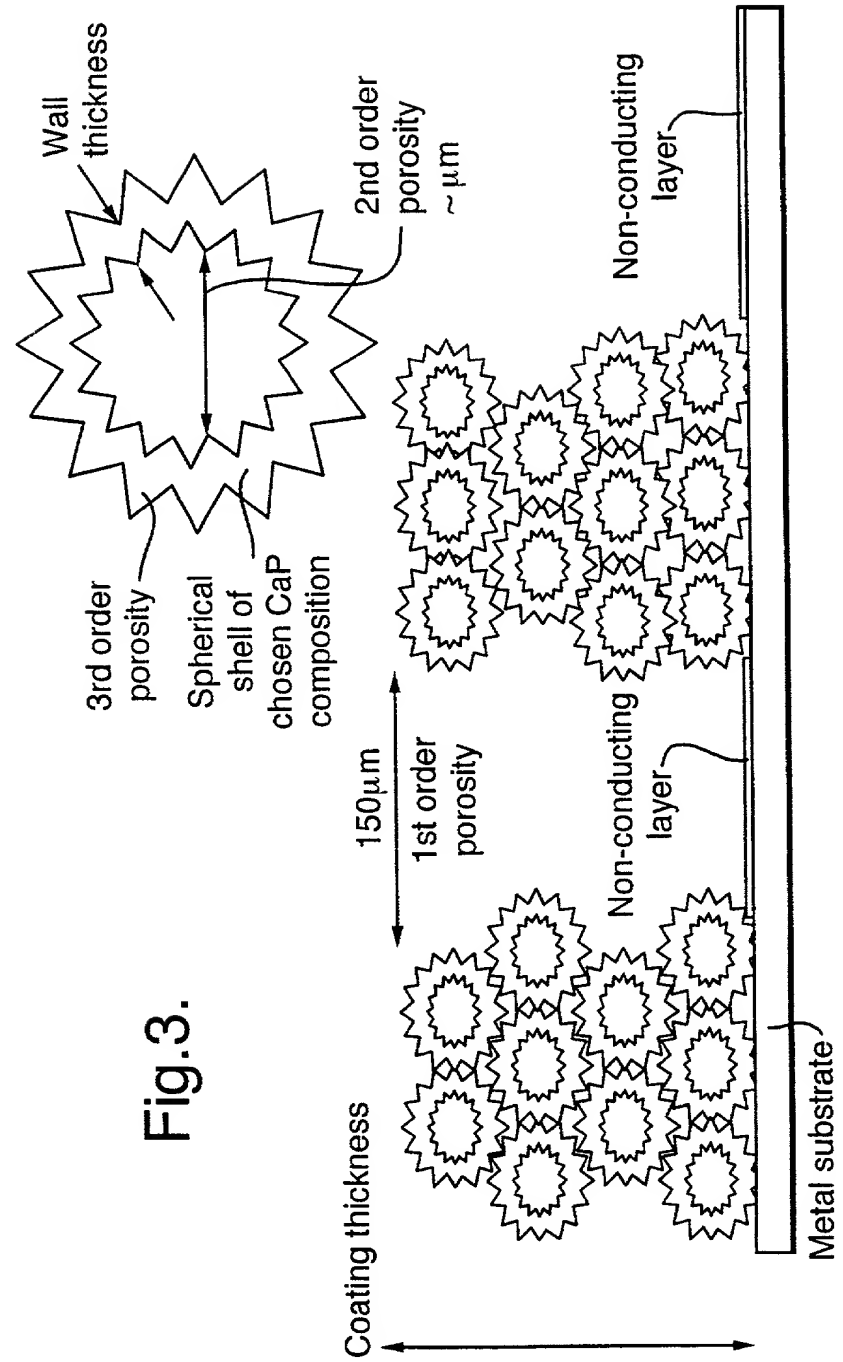

Wall thickness
2nd order porosity ~µm
Non-conducting layer
3rd order porosity
Spherical shell of chosen CaP composition
150µm
1st order porosity
Non-conducting layer
Coating thickness
Metal substrate

Fig.3.

US

12 MAR 2001

09/720411

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to PCT International Applications)	ATTORNEY DOCKET NUMBER

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CALCIUM PHOSPHATE COATED VESICLES

the specification of which (check only one item below):

[] is attached hereto.

[] was filed as U.S. Patent Application Serial Number _ on _, as amended on _ (if applicable).

[X] was filed as a PCT international application number PCT/GB 99/02005 on 25 June 1999, as amended under PCT Article 19 on _ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the applications for which priority is claimed:

PRIOR FOREIGN PATENT APPLICATION(S) AND ANY PRIORITY CLAIMED UNDER 35 U.S.C. §119:

COUNTRY (If PCT Indicate PCT)	APPLICATION NUMBER	DATE OF FILING (Day, Month, Year)	PRIORITY CLAIMED UNDER 35 USC 119
United Kingdom	9813906.6	26 June 1998	[X] YES [ ] NO
			[ ] YES [ ] NO
			[ ] YES [ ] NO
			[ ] YES [ ] NO
			[ ] YES [ ] NO

US

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to PCT International Applications)	ATTORNEY DOCKET NUMBER

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

U.S. APPLICATIONS		STATUS (Check One)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	ABANDONED	PENDING

PCT APPLICATIONS DESIGNATING THE U.S.

PCT APPLICATION NUMBER	PCT FILING DATE	U.S. SERIAL NUMBERS			

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith (List names and registration numbers): Carl R. Schwartz, Reg. No. 29,437; George E. Haas, Reg. No. 27,642; Jean C. Baker, Reg. No. 35,433; David G. Ryser, Reg. No. 36,407

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	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
J. Czernuszka	David Haddow	
DATE: 8.1.2001	DATE: 16.1.2001	DATE

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